

WHAT IS CLAIMED IS:

✓ 1. A reverse transcriptase which has been modified or mutated by at least one way selected from the group consisting of:

- (a) to increase or enhance fidelity;
- (b) to reduce or eliminate misincorporation of nucleotides during nucleic acid synthesis; and
- (c) to decrease or eliminate terminal deoxynucleotidyl transferase activity.

wherein said reverse transcriptase is not derived from a Human Immunodeficiency Virus.

2. The reverse transcriptase of claim 1, wherein said reverse transcriptase is modified or mutated to increase or enhance fidelity.

3. The reverse transcriptase of claim 1, wherein said reverse transcriptase is modified or mutated to reduce or eliminate misincorporation of nucleotides during nucleic acid synthesis.

4. The reverse transcriptase of claim 1, wherein said reverse transcriptase is modified or mutated to decrease or eliminate terminal deoxynucleotidyl transferase activity.

5. The reverse transcriptase 1, wherein said reverse transcriptase is modified or mutated to increase or enhance fidelity, and to reduce or eliminate misincorporation of nucleotides during nucleic acid synthesis.

6. The reverse transcriptase of claim 1, wherein said reverse transcriptase is modified or mutated to increase or enhance fidelity, and to decrease or eliminate terminal deoxynucleotidyl transferase activity.

09808124-031501  
FOSTER-428086

7. The reverse transcriptase of claim 1, wherein said reverse transcriptase is modified or mutated to reduce or eliminate misincorporation of nucleotides during nucleic acid synthesis, and to decrease or eliminate terminal deoxynucleotidyl transferase activity.

8. The reverse transcriptase of claim 1, wherein said reverse transcriptase is further modified or mutated to reduce or substantially reduce RNase H activity.

9. The reverse transcriptase of claim 1, wherein said reverse transcriptase is derived from a virus selected from the group consisting of M-MLV, RSV and AMV.

10. The reverse transcriptase of claim 8, wherein said reverse transcriptase is derived from a reverse transcriptase selected from the group consisting of M-MLV H<sup>-</sup> reverse transcriptase, RSV H<sup>-</sup> reverse transcriptase, AMV H<sup>-</sup> reverse transcriptase and RAV H<sup>-</sup> reverse transcriptase.

11. The reverse transcriptase of claim 9, wherein said modified or mutated M-MLV has a mutation or modification at position Tyr64.

12. The reverse transcriptase of claim 11, wherein Tyr64 is replaced with a tryptophan.

13. The reverse transcriptase of claim 9, wherein said modified or mutated M-MLV has a mutation or modification at position Arg116.

14. The reverse transcriptase of claim 13, wherein Arg116 is replaced with a methionine.

09808124.031501

Sub  
B1

Sub  
B2

sub B3 15. The reverse transcriptase of claim 9, wherein said modified or mutated M-MLV has a mutation or modification at position Lys152.

16. The reverse transcriptase of claim 15, wherein Lys152 is replaced with an arginine.

sub B4 17. The reverse transcriptase of claim 9, wherein said modified or mutated M-MLV has a mutation or modification at position Glu190.

18. The reverse transcriptase of claim 17, wherein Glu190 is replaced with a phenylalanine.

19. The reverse transcriptase of claim 9, wherein said modified or mutated M-MLV has a mutation or modification at position Thr197.

20. The reverse transcriptase of claim 19, wherein Thr197 is replaced with an alanine.

sub B5 21. The reverse transcriptase of claim 9, wherein said modified or mutated M-MLV has a mutation or modification at position Val223.

22. The reverse transcriptase of claim 21, wherein Val223 is replaced with a histidine.

23. The reverse transcriptase of claim 9, wherein said modified or mutated AMV has a mutation or modification at a position selected from the group consisting of Trp25, Arg76, Lys110, Glu149, Thr156, and Met182.

24. The reverse transcriptase of claim 9, wherein said modified or mutated RSV has a mutation or modification at a position selected from the group consisting of Trp25, Arg76, Lys110, Glu149, Thr156 and Met182.

09808124.031501

25. The reverse transcriptase of claim 1, wherein the fidelity of said modified or mutated reverse transcriptase is from about 1.5 to about 50 times that of the unmodified or unmutated reverse transcriptase.

26. The reverse transcriptase of claim 1, wherein the fidelity of said modified or mutated reverse transcriptase is from about 10 to about 50 times that of the unmodified or unmutated reverse transcriptase.

27. The reverse transcriptase of claim 1, wherein the fidelity of said modified or mutated reverse transcriptase is from about 20 to about 50 times that of the unmodified or unmutated reverse transcriptase.

28. The reverse transcriptase of claim 1, wherein the fidelity of said modified or mutated reverse transcriptase is from about 30 to about 50 times that of the unmodified or unmutated reverse transcriptase.

29. The reverse transcriptase of claim 1, wherein the misincorporation rate of said modified or mutated reverse transcriptase is about 50% of the misincorporation rate of the unmodified or unmutated reverse transcriptase.

30. The reverse transcriptase of claim 1, wherein the misincorporation rate of said modified or mutated reverse transcriptase is about 25% of the misincorporation rate of the unmodified or unmutated reverse transcriptase.

31. The reverse transcriptase of claim 1, wherein the misincorporation rate of said modified or mutated reverse transcriptase is about 10% of the misincorporation rate of the unmodified or unmutated reverse transcriptase.

09808124-031501

32. The reverse transcriptase of claim 1, wherein the modification is in the fingers region of the reverse transcriptase.

33. The reverse transcriptase of claim 1, wherein the modification is in the thumb region of the reverse transcriptase.

34. The reverse transcriptase of claim 1, wherein said modified or mutated M-MLV has a mutation or modification at position Phe309.

35. The reverse transcriptase of claim 34, wherein Phe309 is replaced with an asparagine.

36. The reverse transcriptase of claim 1, wherein said modified or mutated M-MLV has a mutation or modification at position Thr197.

37. The reverse transcriptase of claim 36, wherein said Thr197 is replaced with a glutamic acid.

38. The reverse transcriptase of claim 1, wherein said modified or mutated M-MLV has a mutation or modification at position Tyr133.

39. The reverse transcriptase of claim 38, wherein Tyr133 is replaced with an alanine.

40. The reverse transcriptase of claim 1, wherein said modified or mutated AMV has a mutation or modification at a position selected from the group consisting of Trp267 and Ala95.

41. The reverse transcriptase of claim 1, wherein said modified or mutated RSV has a mutation or modification at a position selected from the group consisting of Trp267 and Ala95.

09808124-031501

42. The reverse transcriptase of claim 1, wherein the terminal deoxynucleotidyl transferase specific activity of said modified or mutated reverse transcriptase is less than about 75% of the unmodified or unmutated reverse transcriptase specific activity.

43. The reverse transcriptase of claim 1, wherein the terminal deoxynucleotidyl transferase specific activity of said modified or mutated reverse transcriptase is less than about 50% of the unmodified or unmutated reverse transcriptase specific activity.

44. The reverse transcriptase of claim 1, wherein the terminal deoxynucleotidyl transferase specific activity of said modified or mutated reverse transcriptase is less than about 25% of the unmodified or unmutated reverse transcriptase specific activity.

45. The reverse transcriptase of claim 1, wherein the terminal deoxynucleotidyl transferase specific activity of said modified or mutated reverse transcriptase is less than about 10% of the unmodified or unmutated reverse transcriptase specific activity.

46. The reverse transcriptase of claim 1, wherein the terminal deoxynucleotidyl transferase specific activity of said modified or mutated reverse transcriptase is less than about 1% of the unmodified or unmutated reverse transcriptase specific activity.

47. A vector comprising a nucleic acid molecule encoding the reverse transcriptase of claim 1.

48. The vector of claim 47, wherein said nucleic acid molecule is operably linked to a promoter.

09808124.031501

49. The vector of claim 48, wherein said promoter is selected from the group consisting of a l-P<sub>L</sub> promoter, a *tac* promoter, a *trp* promoter, and a *trc* promoter.

50. A host cell comprising the vector of claim 47.

51. A method of producing a reverse transcriptase, said method comprising:

- (a) culturing the host cell of claim 50;
- (b) expressing said nucleic acid molecule; and
- (c) isolating said reverse transcriptase from said host cell.

52. The method of claim 51, wherein said host cell is *Escherichia coli*.

53. A method for reverse transcription of one or more first nucleic acid molecules, said method comprising:

- (a) mixing one or more nucleic acid templates with one or more reverse transcriptases of claim 1; and
- (b) incubating the mixture of (a) under conditions sufficient to make one or more first nucleic acid molecules complementary to all or a portion of said one or more templates.

54. The method of claim 53, wherein said nucleic acid template is a messenger RNA molecule or a population of mRNA molecules.

55. The method of claim 53, further comprising incubating said one or more first nucleic acid molecules under conditions sufficient to make one or more second nucleic acid molecules complementary to all or a portion of said one or more first nucleic acid molecules.

09808124-031501  
FOSTERO-4280860

56. A cDNA molecule made according to the method of claim 53.
57. A cDNA molecule made according to the method of claim 55.
58. A method for amplifying one or more nucleic acid molecules, said method comprising:
- (a) mixing one or more nucleic acid templates with a reverse transcriptase of claim 1 and one or more DNA polymerases; and
  - (b) incubating the mixture of (a) under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said one or more templates.
59. A method for sequencing one or more nucleic acid molecules, said method comprising:
- (a) mixing one or more nucleic acid molecules to be sequenced with one or more primers, a reverse transcriptase of claim 1, one or more nucleotides and one or more terminating agents;
  - (b) incubating the mixture of (a) under conditions sufficient to synthesize a population of molecules complementary to all or a portion of said one or more molecules to be sequenced; and
  - (c) separating said population from other molecules in said population to determine the nucleotide sequence of all or a portion of said one or more molecules to be sequenced.

09808124-031501  
FOSTER 42780860



60. A kit for use in reverse transcription, amplification or sequencing of a nucleic acid molecule, said kit comprising one or more reverse transcriptases of claim 1.

61. The kit of claim 60, further comprising one or more components selected from the group consisting of one or more nucleotides, one or more DNA polymerases, one or more buffers, one or more primers, and one or more terminating agents.

62. The kit of claim 61, wherein said terminating agent is a dideoxynucleotide.

add  
Bb

09808124-031501